



## A Method to Obtain a Well-Defined Fraction of Respirable *para*-Aramid Fibers

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The aim of this study was to develop a preparation method for *p*-aramid in order to obtain respirable fibers for further biological studies. The experiments were carried out using samples of Twaron (type 1095/charge no. 172633), kindly provided by AKZO/Arnhem. We suspended 2 g of *p*-aramid pulp in 800 ml distilled water; in some experiments we added 0.25 or 0.125% ethanol (Prolabo, 99.85%) to change the degree of floatability of the fibers. Suspensions were stirred at 500 rpm for 1, 3, or 15 hr at room temperature, followed by a settling step (1 or 5 hr). After settling, two clear-cut phases were distinguishable on the basis of their color: the upper phase was lighter than the bottom phase. The upper phase volume increased with settling time, and after 5 hr reached 27–35% (range of 12 experiments) of the total volume (depending on stirring time and ethanol concentration). The average volume of the upper phase was 232 ml  $\pm$  32 ml (mean  $\pm$  SD of 16 experiments) independently of the experimental conditions. For size analyses, samples were taken at about 0.5 cm beneath the surface of the settled suspensions.

We determined the weight of the *p*-aramid fraction in the upper phase by differential gravimetric analysis using Nuclepore membranes (0.4  $\mu$ m pore size); 50 ml of the upper phase was filtered per membrane followed by rinsing with distilled water (2  $\times$  50 ml). The weight of the particles recovered on the membrane was 0.04  $\pm$  0.01 mg/ml (mean  $\pm$  SD of seven experiments), ranging between 0.032 mg/ml and 0.048 mg/ml. The upper phase (250 ml sampled) represented around 0.5% of the weight of the starting material under our experimental conditions.

The homogeneity of the dispersion of *p*-aramid fibers on the Nuclepore membranes was checked by scanning electron microscopy (SEM) analyses (JEOL, JSM-840). Morphological analysis of the fibers in the upper phase shows that *p*-aramid fibers have a ribbonlike shape; they are sometimes twisted, with a variable diameter along the fiber (Figs. 1 and 2). Moreover, fibers are curled; for these two reasons diameter and length are both difficult to measure accurately, and the size should

be considered as an estimation. SEM analysis under an angle of 60° at various magnifications indicated that fibers were lying mostly flat on the filter (Fig. 2).

Size distribution of the *p*-aramid fibers was determined by SEM and transmission electron microscopy (TEM; JEOL, JM-100CX-II). TEM grid preparations were performed as previously described (1). Briefly, carbon-coated Nuclepore membranes (0.4  $\mu$ m; filter diameter 47 mm) were deposited on a clean filter holder (Millipore/XX2004720); 150  $\mu$ l of the upper phase was filtered under vacuum, and the filter was rinsed with distilled water (2  $\times$  50 ml). We removed the filter, dried it in an oven at 60°C for 30 min on petri slides (Millipore/PD1504700), covered it with a second carbon layer, and transferred particles onto 200-mesh TEM copper grids as follows: for each membrane, three grids were placed on clean paper (Prolabo, 45 mm diameter, 08.312.706) deposited on a filter holder as used for the preceding filtration. Part of each carbon-coated membrane (about 1 cm<sup>2</sup>) was deposited on the grids and dissolved by chloroform (Prolabo) under vacuum. In some cases 0.5 cm<sup>2</sup> of the remaining membrane was covered with gold for SEM analysis. We counted and classified the fibers according to the general counting rules for asbestos fibers (2). At least 100 fibers were counted per grid at 10,000 $\times$  magnification. Split fibers were considered as one fiber; overlapping fibers were counted separately after accurate analysis at higher magnifications. Bundles were counted at low magnification (600 $\times$ ). Fiber length and diameter were determined at 10,000 $\times$  magnification. For a partly split fiber, we always measured the longer branch ( $L_{max}$ ). In case of variations in diameter for one fiber, the largest diameter was noted ( $D_{max}$ ). Percentages of particles with aspect ratios equal or more than 3:1 and aspect ratios equal or more than 5:1 were calculated.

We conducted statistical analyses to explore variations in fiber metrology. The *t*-test was used to explore two-sample differences, i.e., grid to grid or trial to trial comparisons. The *F*-test was used to explore overall differences in stirring and settling conditions or ethanol. The mean

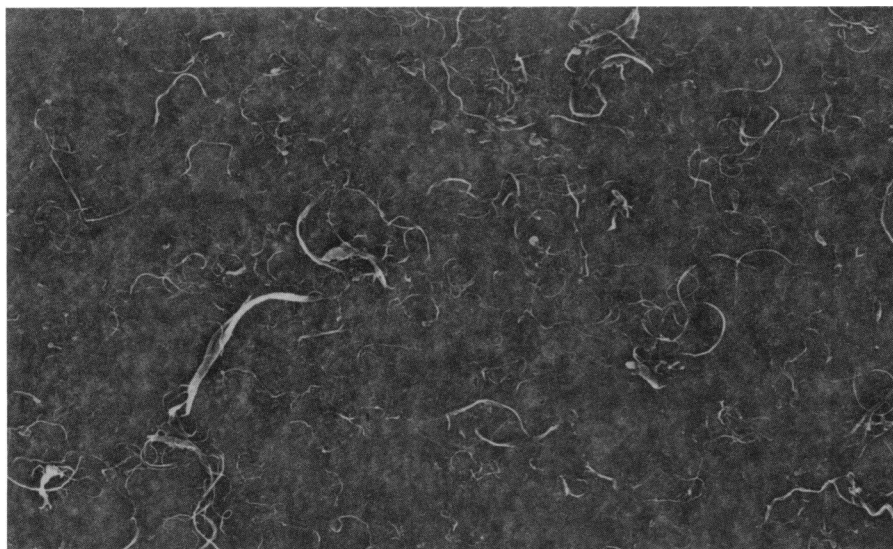
We developed a preparation method to obtain respirable-sized fractions of *para*-aramid fibers. The procedure, based on floatability, consists of stirring and subsequent settling of *p*-aramid pulp in distilled water. Two distinct phases are obtained, with small fibers in the upper part of the suspension, which represents about 33% of the total volume. Optimal results were obtained when 2.0 g pulp was stirred for 15 hr in 800 ml distilled water containing 0.125% ethanol and settled for 5 hr. The mass yield ranged between 0.4 and 0.6%, more than 90% of the particles had an aspect ratio  $\geq 3:1$ . The mean fiber length was about 6  $\mu$ m, and the mean fiber diameter was about 0.4  $\mu$ m as determined by transmission and scanning electron microscopy. The number of fibers obtained was  $4 \times 10^6$  fibers/ $\mu$ g under our standard conditions. **Key words:** electron microscopy, floatability, metrology, *para*-aramid fibers, respirable fibers. *Environ Health Perspect* 101:414–416(1993)

length and mean diameter values are not significantly different between grids of a given trial and between trials except for fiber diameter after 3 hr of stirring and 1 hr of settling (grids a5-b1, Table 1). This suggests that the deposition of fibers from a given filtration phase is homogeneous on the Nuclepore membrane surface and allows the comparison between trials using only one electron grid, except for stirring and settling time  $\leq 3$  hr and 1 hr, respectively. Under these conditions, it is possible that the dispersion of preexisting fibers is incomplete or that settling of larger fibers is insufficient. Mean size-distribution values were not statistically different from one trial to another for stirring  $> 1$  hr.

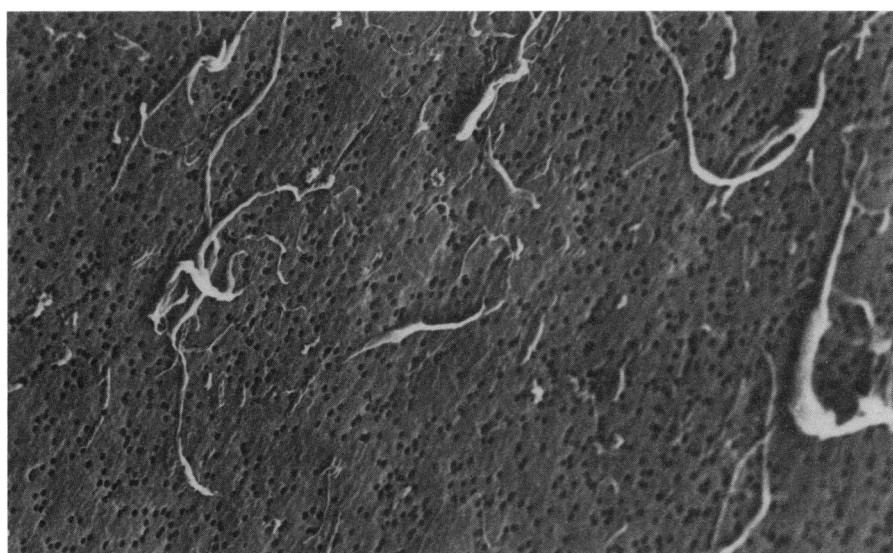
Stirring for 3 or 15 hr significantly reduced both length and diameter in comparison with 1 hr (grids b4, b5, d2 versus a5, b1 and c3 versus c8; Table 1); no significant differences were seen in mean

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**Figure 1.** Scanning electron micrograph of *p*-aramid fibers in the upper phase. Experimental conditions: 3 hr stirred, 5 hr settled, 0.125% ethanol (500 $\times$ ).



**Figure 2.** Scanning electron micrograph of *p*-aramid fibers in the upper phase. Experimental conditions: 15 hr stirred, 5 hr settled, 0.125% ethanol (3100 $\times$ ).

fiber size between 3 hr and 15 hr of stirring (grid a5, b1 versus c3, c8), indicating that the dispersion of fibers in the pulp likely takes place during the first 3 hr of stirring.

The addition of ethanol was associated with a significant decrease in fiber diameter (Table 1) after 1 hr of settling but not after 5 hr of settling. Settling time had little effect on fiber size; only small differences between 1 hr and 5 hr of settling were observed. The difference after 1 hr of stirring (0.125% ethanol) may be due to the heterogeneity of the suspension, as discussed earlier. The difference after 3 hr of stirring observed in 0.25% ethanol may be due to the presence of ethanol; differences in ethanol concentrations are associated with variation in fiber dimensions after 1 hr of settling but not after 5 hr of settling.

Figure 3 is a histogram of the size distribution of the fibers counted in the upper phase of all grids and shows that most of the fibers counted were of respirable size. Under our experimental conditions, there was a large range of dimensions, from 0.6  $\mu\text{m}$  to 25  $\mu\text{m}$  in length and from 0.1  $\mu\text{m}$  to 2.5  $\mu\text{m}$  in diameter. A mean length of  $6 \pm 6 \mu\text{m}$  was found, and the diameter was approximately  $0.4 \pm 0.4 \mu\text{m}$ ; the geometric means were 4.2  $\mu\text{m}$  and 0.29  $\mu\text{m}$ , respectively. Median values were 3.8  $\mu\text{m}$  for length and 0.5  $\mu\text{m}$  for diameter, modal numbers were 1.9  $\mu\text{m}$  for length and 0.13  $\mu\text{m}$  for diameter. More than 90% of the particles in the upper phase were fibers (as defined by an aspect ratio  $\geq 3$ ) (Table 1). Fibers ranged from 5.3 to 9.3  $\mu\text{m}$  in mean length and 0.30 to 0.53  $\mu\text{m}$  in mean diameter. The percentage of potentially carcinogenic fibers corresponding to the Stanton criteria (3) was around 5% of particles  $\geq 8 \mu\text{m}$  of length and  $\leq 0.25 \mu\text{m}$  in diameter.

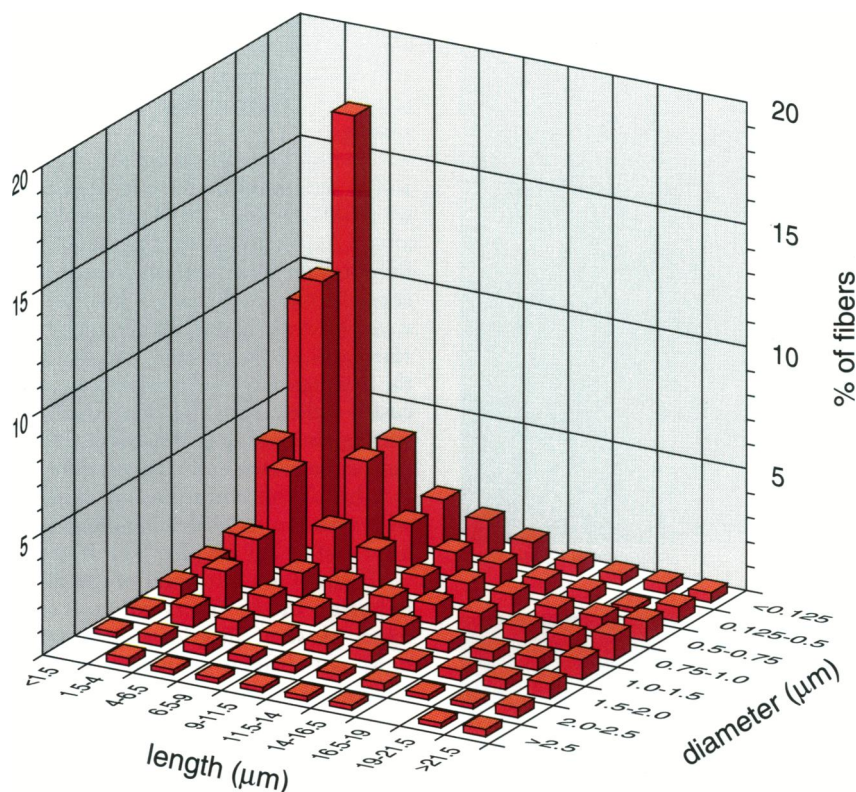
**Table 1.** Characteristics of *p*-aramid fibers recovered in the upper phase

Grid	Hours stirred	Hours settled	% Ethanol	Aspect ratio $\geq 3:1$ (%)	Aspect ratio $\geq 5:1$ (%)	Length $\geq 8 \mu\text{m}$ $\times$ diam $\leq 0.5 \mu\text{m}$	Length $\geq 4 \mu\text{m}$ $\times$ diam $\leq 1.5 \mu\text{m}$	Fibers/ml ( $\times 10^7$ )	No. of fibers counted	Length ( $\mu\text{m}$ ) <sup>a</sup>	Diameter ( $\mu\text{m}$ ) <sup>a</sup>
a1	3	1	0.25	94	91	6	32	8.29	105	$5.3 \pm 6.1$	$0.30 \pm 0.33$
a3	3	5	0.25	97	94	5	42	6.35	139	$6.4 \pm 5.9$	$0.41 \pm 0.43$
a5	3	1	0.125	99	94	3	39	8.15	176	$5.7 \pm 6.0$	$0.32 \pm 0.37$
b1	3	1	0.125	93	87	3	37	7.91	164	$5.8 \pm 6.5$	$0.40 \pm 0.40$
b2	3	5	0.125	99	95	4	41	7.91	157	$6.0 \pm 5.9$	$0.39 \pm 0.40$
b3	3	5	0.125	95	88	5	46	8.01	173	$6.4 \pm 6.1$	$0.44 \pm 0.47$
b4	1	1	0.125	96	89	4	46	10.59	192	$6.6 \pm 6.0$	$0.46 \pm 0.47$
b5	1	1	0.125	98	92	7	44	7.48	167	$7.5 \pm 6.9$	$0.45 \pm 0.47$
d2	1	1	0.125	96	92	6	47	10.88	177	$9.3 \pm 7.6$	$0.53 \pm 0.51$
c3	15	1	0.125	95	91	3	38	12.15	152	$5.8 \pm 5.7$	$0.39 \pm 0.43$
c4	15	5	0.125	93	85	8	31	4.16 <sup>b</sup>	137	$5.7 \pm 6.5$	$0.38 \pm 0.39$
c5	15	5	0.125	97	93	4	41	15.77	150	$6.1 \pm 5.9$	$0.38 \pm 0.44$
d1	15	5	0.125	90	86	5	41	14.06	209	$5.9 \pm 5.8$	$0.43 \pm 0.43$
d4	1	5	0.125	99	93	3	48	14.68	168	$7.7 \pm 6.6$	$0.49 \pm 0.49$
b9	3	1	0	94	90	3	45	17.92	151	$6.4 \pm 5.7$	$0.46 \pm 0.49$
c6	3	5	0	95	86	6	42	10.09	185	$6.7 \pm 6.5$	$0.45 \pm 0.44$
c8	15	1	0.125	95	90	5	44	7.74	180	$7.0 \pm 6.6$	$0.46 \pm 0.47$

<sup>a</sup>Means  $\pm$  SD.

<sup>b</sup>This low yield was likely due to a technical error and was not included in the calculation.





**Figure 3.** Length and size distribution of *p*-aramid fibers obtained in standard conditions (all grids considered).

The number of fibers recovered in the upper phase ranged between  $6.3 \times 10^7$  and  $17.9 \times 10^7$ /ml. This number of fibers was  $2.5 \times 10^6$ /μg of the material contained in the upper phase, which means that an order of magnitude of  $10^{10}$  fibers may be released from 1 g of *p*-aramid pulp under our experimental conditions. The most reproducible results were obtained with an ethanol concentration of 0.125%, 15 hr of stirring, and 5 hr of settling. Under these conditions a mean of  $4.4 \times 10^6$ /μg was found. Although the mass yield of fibers was low, one can conclude from these data that numerical yield of the respirable *p*-aramid fibers is high. Additionally, the number of bundles was negligible with respect to the number of fibers, in spite of the large contribution to fiber mass. The proportion of bundles observed at 600× magnification ranged between  $5 \times 10^4$  to  $20 \times 10^4$ /ml, which represents approximately 0.03–0.3% with respect to the number of respirable fibers.

As recently demonstrated in an interlaboratory study on asbestos fiber counting (4), underestimation in *p*-aramid fiber number cannot be completely excluded. However, in our TEM analysis at high magnification, very small fibers were detected, and the numerical concentrations of fibers in different trials did not highly differ; no decrease of fibers/milliliter in the upper phase during sedimentation from 1 to 5 hr was established. Moreover, range in

fiber mass is small, and there is a good correlation between mass of particles weighed on the filter and mass calculated from the numerical concentrations. If the *p*-aramid fibers are considered as a half cylinder (considering its ribbonlike shape), a fiber 0.4 μm in diameter and 6 μm in length would have a mass of  $5.4 \times 10^{-10}$  mg. As we obtained, on average,  $10^8$  fibers/ml, the calculated corresponding mass is 0.054 mg/ml. This is close to the range of 0.032 to 0.048 mg/ml determined by gravimetric measurements, suggesting that our results can be considered a good estimation of the actual number of fibers present in the upper phase.

The fiber preparation used in this experiment has been previously used by Dunnigan et al. (5), but no detailed description of both method and results has been presented. Our findings are different in terms of average length and diameter: Dunnigan et al. (5) reported 2.72 μm length (versus our 6 μm) and 0.138 μm diameter (versus our 0.4 μm). However, the percentage of fibers  $\leq 5$  μm in length and  $\leq 0.25$  μm in diameter was not highly different. Several explanations can be given: first, the fibers were of different origin (Dunnigan et al. used Kevlar, whereas we used Twaron); second, the raw pulp may arise from different sources; and third, differences in analytical electron microscopy method might exist, but Dunnigan et al. (5) gave no information on these parameters.

In summary, the floatation method is a convenient procedure (simple, fast) for obtaining respirable *p*-aramid fibers. It appears to be reproducible in yielding a well-defined quantity (number of fibers, fiber mass) and type (length, diameter, aspect ratio) of fibers and can produce fibers of toxicological relevance that can be used for *in vitro* or some *in vivo* studies involving instillation or intracavity studies. Further experiments could be made to determine whether large amounts of fibers can be obtained for inhalation studies.

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